7/7/98

DATA EVALUATION RECORD
§ 72-1(C) -- ACUTE LC_{50} TEST WITH A COLDWATER FISH

1. **CHEMICAL:** EPTC Shaughnessey #: 041401

2. **TEST MATERIAL:** Technical Lot# 518-713, BR-83-50 Purity: 99 %

3. **CITATION:**
   Authors: Forbis, A.D.,C.M. Thompson,P.M. Buckler and L.D.Johnson.
   Title: Acute toxicity of EPTC to rainbow trout (*Salmo gairdneri*)
   Study Completion Date: July 28, 1983
   Laboratory: ABC Laboratories, Inc., Columbia, MO
   Laboratory Report ID: Static Bioassay Report 30652
   Sponsor: PPG Industries Incorporated, Barberton, OH
   MRID No.: 001312-72 (Blowback ID 42980202)

4. **REVIEWED BY:** William S. Rabert, Biologist, ERB III, EFED

   Signature: William S. Rabert **Date:** 7/6/98

5. **APPROVED BY:** Harry Craven, Senior Scientist, ERB III, EFED

   Signature: Harry Craven **Date:** 7/7/98

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Oncorhynchus mykiss*
Age or Size of Test Organism:	Juveniles (ca. 2.9 cm)
Definitive Test Duration:	96 hours
Study Method:	Static
Type of Concentrations:	Nominal conc.

7. **CONCLUSIONS:** The acute 96-hour LC_{50} toxicity study is scientifically sound, and fulfills the requirement for a coldwater fish acute LC_{50} test using rainbow trout. Volatility of EPTC during the 96-hour test may have affected the LC_{50} value, but based on other studies volatility is unlikely to alter the toxicity category. This study indicates that EPTC is practically slightly toxic to coldwater fish.

   Results Synopsis: Based on nominal concentrations:

   LC_{50}: 21 mg/L 95% C.I.: 10 - 32 mg/L

   NOEL: 3.2 mg/L Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

   A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The rainbow trout used in this study were smaller than the recommended size, but the smaller size probably had no adverse effect on the test results.

2. Test vessels were placed in a water bath, but test temperatures were measured only every 48 hours and not measured continuously or the minimum of at least every 6 hours.

3. Measurements of temperature, pH, and dissolved oxygen were made every 48 hours rather than daily.

4. The well water was analyzed and contained some contaminants that exceed the EPA/ASTM recommended water quality characteristics (e.g., iron 56 ppb and lead 8.3 ppb). Fluoride levels were not measured at all (see attachment). Reconstitution of the water may have removed these contaminants, but water quality measurements were not reported for the reconstituted, soft dilution water used in the test.

10. SUBMISSION PURPOSE: Old study listed for Reregistration.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the rainbow trout (*Oncorhynchus mykiss*)	Rainbow trout *Oncorhynchus mykiss*
Mean Weight: 0.5-5 g	Mean: 0.34 g
Mean Standard Length: Longest not > 2x shortest	SL: Mean 2.9 cm; Range 2.6 to 3.2 cm
Supplier	Spring Creek Trout Hatchery Lewistown, Montana
All fish from same source?	Yes

Guideline Criteria	Reported Information
All fish from the same year class?	Not stated, but the size indicates it is so.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: Minimum 14 days	At least 14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Fish not were fed 48 hours prior to nor during the test.
Pretest Mortality < 3% mortality 48 hours prior to testing	No mortality during 14-day period prior to test

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Soft reconstituted well water.
Does water support test animals without observable signs of stress?	Observation of sublethal effects was not reported.
Water Temperature: 12°C	temperature ranged from 12 - 13 °C; Measured only on Days 0, 2, and 4.
pH: Prefer 7.2 to 7.6	pH range: 7.0 - 7.3
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	D.O. range: 7.9 to 8.9 mg/L; 71 to 80 % saturation
Total Hardness: Prefer 40 to 48 mg/L as $CaCO_3$	Range: 40 - 45 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Glass aquaria 5 gallon 15 L, water depth was not reported.
Type of Dilution System Must provide reproducible supply of toxicant	Static test; N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate ???? loading for 12°C Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.23 g/L 0.34 g x 10 fish/ 15 L = 0.23 g/L
Photoperiod: 16 hours light, 8 hours dark	16 hours light: 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	 Solvent: acetone Maximum conc.: 0.21 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	The LC50 for the range-finding test was > 10 < 100 mg/L.
Nominal Concentrations of Definitive Test: Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, solvent control, and nominal concentrations of: 3.2, 5.6, 10, 18, and 32 mg/L
Number of Test Organisms: Minimum 10 per level, may be divided among containers	1 replicate of 10 fish

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	"Random assignment".
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and every 48 hours in the high, medium, and low doses and in the controls	temperature measured every 48 hours; but not continuously. pH and DO measured every 48 hours.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Test concentrations were not measured.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	N/A
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0 %
Raw data included?	Yes.
Signs of toxicity (if any) were described?	Yes.

Mortality and sublethal effects:

Treatment-related effects were seen at exposure levels of 5.6 mg/L and above.

5.6 mg/L: Six fish observed on bottom at 24 hours; normal at 48 through 96 hours.
10 mg/L: All fish affected throughout the test: ten fish on bottom at 24 hours; at 96 hours 2 fish were darkened; 1 fish at surface, and 7 fish on the bottom.
18 mg/L: All fish affected throughout the test: 3 fish with loss of equilibrium and dark coloration; and 7 fish on the bottom and darkened; at 96 hours 3 fish dead, 2 fish with loss of equilibrium and darkened, and 5 fish on bottom and darkened.
32 mg/L: All fish dead at 24 hours.

Concentration (ppm)		Number of Fish	Cumulative Percent Number of Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	--	10	0	0	0	0
Solvent Control	--	10	0	0	0	0
3.2	--	10	0	0	0	0
5.6	--	10	0	0	0	0
10	--	10	0	0	0	0
18	--	10	0	0	0	30
32	--	10	100	100	100	100

Other Significant Results: None

B. Statistical Results

Method: Binomial test (nominal conc.)

96-hr LC_{50}: 20.52 mg/L ai 95% C.I.: 10 - 32 mg/L ai

Probit Slope: Not Reported NOEC: 3.2 mg/L ai

13. VERIFICATION OF STATISTICAL RESULTS

The test concentrations were not measured.

Parameter	Result

Binomial Test LC_{50} (C.I.)	21 (10 - 32) mg/L
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	3.2 mg/L

14. REVIEWER'S COMMENTS:

Calculated toxicity values were the same as reported by the authors. Only one test concentration had partial mortality of the 10 fish.

The water solubility for EPTC is 370 ppm at 20 °C. No data are available on the octanol-water partition coefficient, but based on the water solubility, EPTC is unlikely to sorb in significant amounts to organic matter.

EPTC is volatile with a vapor pressure of 1.6×10^{-2} mm Hg, hence stable EPTC concentrations in test solutions is a concern. The vapor losses from test solutions appear to be limited based on measured test concentrations in 7 aquatic studies. EPTC losses were a mean of 4 percent and a range of 2 to 5 percent in a 48-hour daphnid test, 18 percent with a range of 0 to 37 percent during the 72-hour renewal periods in a duckweed test, and means of 37, 37, 38, 46 and 56 percent with ranges of 29 to 61 percent in algal tests. The higher losses in the algal studies probably reflect increased volatility due to low volume (100 ml) to surface area and shaking action from a rotating table surface. Since the volume of test solution in fish studies is about 15 liters, test solutions are not agitated, and is tested at a lower test temperature, volatility is expected to be less than 25 percent during the 96-hour test as suggested by measurements in the daphnid and duckweed studies.

The description of water quality characterization for ABC's well water is incomplete (i.e., measurements of some common contaminants were not reported, such as boron and fluoride. Concentrations of some metals reported in the well water exceed EPA/ASTM recommended concentrations (e.g., iron 56 ppb and lead 8.3 ppb). The dilution water was reconstituted soft water which suggests that the contaminants would have been removed from the test solutions.

William Rabert EPTC Rainbow Trout Static 96-hour LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
32	10	10	100	9.765625E-02
18	10	3	30	17.1875
10	10	0	0	9.765625E-02
5.6	10	0	0	9.765625E-02
3.2	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 10 AND 32 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 20.52643

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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